

**ANTIBACTERIAL AND PROBIOTIC PROPERTIES OF LACTIC  
ACID BACTERIA ISOLATED FROM BEE BREAD OF  
STINGLESS BEE FARM IN MALAYSIA**

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## AUTHOR DECLARATION

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I hereby declare that the work in this dissertation is my own except for quotation and summaries which have been duly acknowledged.

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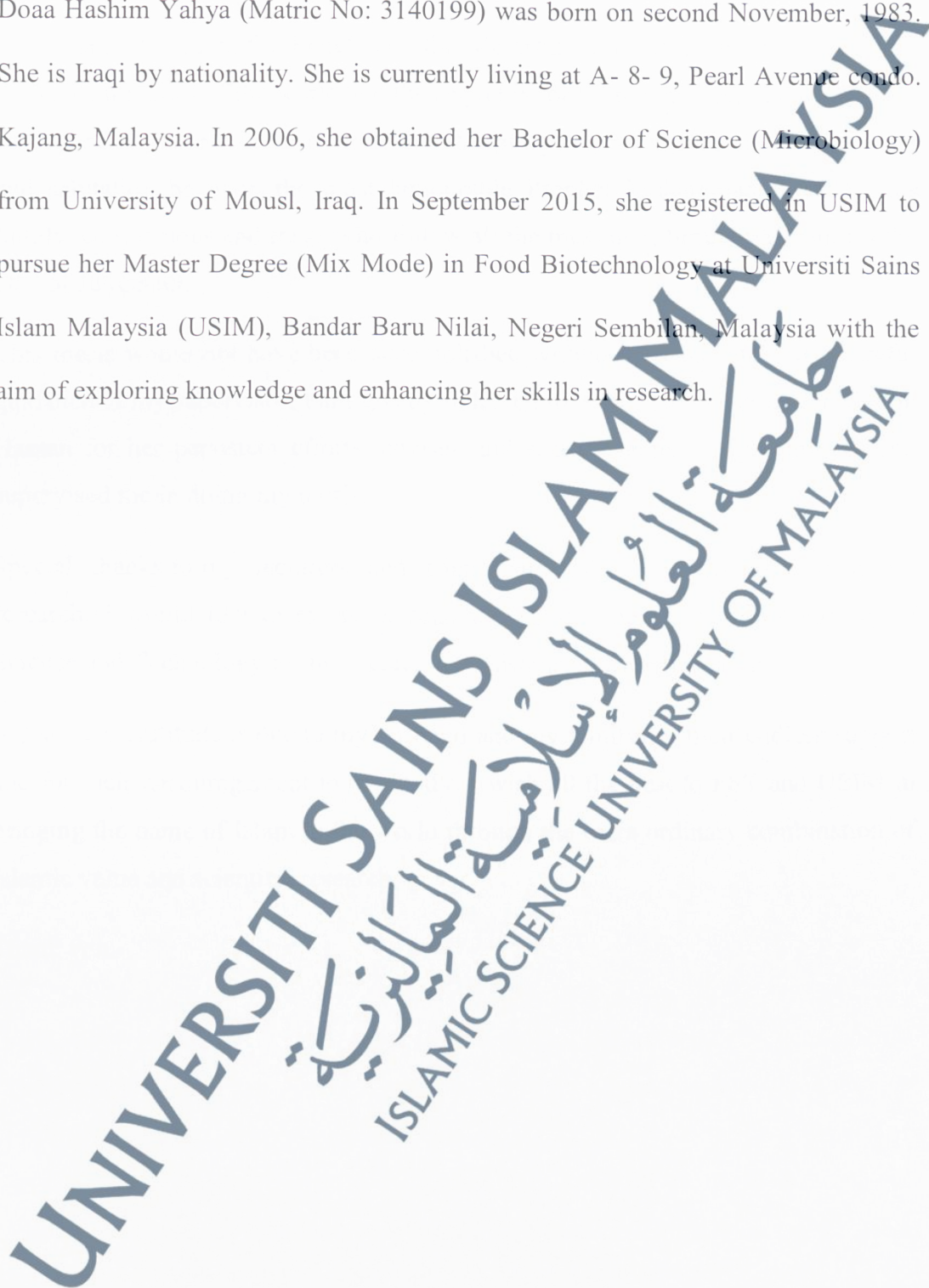
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## ABSTRAK

Terdapat kekurangan maklumat berkaitan dengan jenis bakteria asid laktik (LAB) yang terdapat di ladang lebah kelulut di Malaysia, termasuk ciri-ciri antimikrob dan probiotiknya. Roti lebah (*bee bread*) yang bersifat asid adalah kaya dengan nutrien, dan mungkin menyokong pertumbuhan banyak mikroorganisma termasuk LAB. Tujuan kajian ini adalah untuk mengasingkan bakteria asid laktik daripada roti lebah kelulut yang diperolehi daripada pelbagai spesies dan sumber lebah kelulut. Isolat LAB itu telah dinilai untuk aktiviti anti-bakterianya terhadap tiga bakteria patogenik (*Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 dan *Escherichia coli* ATCC 25922), sifat-sifat probiotiknya, iaitu toleransi terhadap pH yang rendah, keupayaannya untuk terus hidup dalam 0.1 hingga 0.5% hempedu-garam, dan ketahanannya terhadap antibiotik. Pra-pengayaan roti lebah dalam kaldu MRS memudahkan pengasingan lima belas jenis LAB di atas agar MRS dengan 0.8% CaCO<sub>3</sub> berbanding dengan agar jus tomato dengan 0.8% CaCO<sub>3</sub>. Isolat tersebut terbukti mengandungi LAB melalui ujian katalase negatif dan Gram positif. Isolat LAB menunjukkan aktiviti perencatan signifikan ( $p < 0.05$ ) terhadap bakteria sasaran. LAB yang diasingkan daripada *Thorassica* spp, *Itama* spp and *Terminata* spp dari Negeri Sembilan (NS) adalah lebih berkesan terhadap *S. Typhimurium* dan *E. coli*, manakala LAB yang diasingkan dari Sungai Merab (SM) menunjukkan aktiviti perencatan tertinggi terhadap *S. aureus*, seperti yang ditunjukkan oleh diameter zone perencatan: *S. aureus* (14.0 hingga 25.8 mm), *S. Typhimurium* (23.1 hingga 37.6 mm) dan *E. coli* (17 hingga 39.6 mm) selepas pengeraman selama 24 jam pada suhu 37°C melalui kaedah agar dwi tindihan. Supernatan semua isolat LAB menunjukkan aktiviti anti-bakteria yang sangat baik terhadap semua bakteria sasaran pada kadar 50% daripada supernatant bebas sel (CFS). Kehilangan aktiviti anti-bakteria telah diperhatikan selepas pelarasan supernatan kepada pH 5, 6, 7 dan 8. Semua isolat LAB dapat bertahan dalam 0.1 hingga 0.5 % (w/v) hempedu lembu-garam dan pH 2 hingga 4. LAB yang diasingkan daripada roti lebah SM lebih bertahan terhadap pH tinggi dan hempedu-garam daripada LAB yang diasingkan daripada roti lebah NS. Semua isolat LAB mempunyai ketahanan terhadap vancomycin (5µm) dan streptomycin (10 µm); LAB dari roti lebah SM mempunyai ketahanan terhadap gentamycin (10 µm), manakala LAB dari roti lebah NS adalah kurang bertahan. Semua isolat LAB

tersebut adalah sensitif terhadap tetracycline (30  $\mu\text{m}$ ), penisilin G (10  $\mu\text{m}$ ) dan chloramphenicol (30  $\mu\text{m}$ ). Maka, boleh disimpulkan bahawa roti lebah kelulut yang diperolehi daripada pelbagai sumber mengandungi LAB dengan aktiviti antimikrob yang baik terhadap patogen sasaran, tetapi mempunyai sifat-sifat probiotik yang berubah-ubah, dan kurang daya ketahanan terhadap sesetengah antibiotik.

Kata kunci: *Roti lebah, asid laktik, sifat probiotik, aktiviti antimikrob, kelulut*



## ملخص البحث

المعلومات المتوفرة لأنواع بكتريا حامض اللبنيك الموجودة في ماليزيا في غذاء النحل لنوع النحل الذي يسمى stingless bee مع الفعالية المضادة للجراثيم و الخصائص البكتيرية المهمة قليلة. غذاء النحل غني المواد الغذائية. وهو حامضي بطبيعته ما يعطيه امكانية دعم انواع من الكائنات المجهرية للنمو فيه بما فيها بكتريا حامض اللبنيك. كان الهدف من هذه الدراسة لعزل بكتريا حامض اللبنيك من غذاء النحل لنوع النحل stingless bee من مصادر مختلفة في ماليزيا. قيمت هذه العزلات في الخصائص البكتيرية المهمة كتحمل درجة حامضية قليلة و امكانية البقاء في %0.5 - 0.1 من املاح الصفراء وايضا المقاومة لبعض المضادات الحيوية و فعاليتها المضادة لثلاثة من الجراثيم المرضية ( *Staphylococcus aureus* ATCC25923, *Salmonella typhimurium* ATCC13311 and *Escherichia coli* ATCC25922). وجد ان وسط (MRS agar) مع %0.8 كالسيوم كاربونات يعمل على تسهيل عزل بكتريا حامض اللبنيك مقارنة بوسط tomato juice agar مع كالسيوم كاربونات. هذه العزلات اكد على انها بكتريا حامض اللبنيك بانها سالبة للكيتالو وموجبة لصبغة كرام. عزلات هذه البكتريا اظهرت نشاطا مميزا ( $p < 0.05$ ) مثبطا ضد البكتريا المرضية، بكتريا حامض اللاكتيك المعزولة من انواع النحل *Thorasica spp*, *Itama spp* and *Terminata spp* المعزولة من منطقة Negari Sembilan (NS) كانت اكثر فعالية ضد *S. typhimurium* و *E. coli* بينما بكتريا حامض اللاكتيك المعزولة من نوع النحل *Thorasica spp* المعزولة من منطقة (SM) Sungai Merab اظهرت فعالية مثبطة اكبر ضد *S. aureus* حيث وجد ذلك في قطر درجة منع النمو حيث كان (mm 37.6-23.1) لك *S. typhimurium* و (mm 25.8-14.0) ل *S. aureus* و (47 to 39.6 mm) ل *E. coli* بعد 24 ساعة من التحضين بدرجة حرارة 37 درجة سيليزية بواسطة dual agar overlay method. المستخلص الخلوي لجميع عزلات بكتريا حامض اللبنيك اظهرت فعالية ممتازة ضد البكتريا المرضية حتى %50. فقدان الفعالية المضادة للجراثيم لوحظت بعد تغيير درجة حموضة المستخلص البكتيري ل 8, 7, 6, 5. كل عزلات بكتريا حامض اللبنيك كان لها القدرة على امكانية النمو عند %0.5-0.1 من املاح الصفراء ودرجة حامضية تصل الي 4-2. كل بكتريا حامض اللاكتيك المعزولة من غذاء النحل من منطقة SM كانت اكثر مقاومة لدرجة الحموضة العالية ولا ملاح الصفراء من البكتريا المعزولة من غذاء النحل من منطقة NS. كل بكتريا حامض اللاكتيك كانت مقاومة ل vancomycin (5µm) و streptomycin (10 µm) البكتريا المعزولة من غذاء النحل من منطقة SM كانت مقومة ل gentamycin (10 µm) بينما البكتريا المعزولة من غذاء النحل من منطقة NS كانت اقل مقاومة. كل بكتريا حامض اللاكتيك كانت حساسة ل tetracycline (30 µm), penicillin G (10 µm) و chloramphenicol (30 µm). بعد هذه الدراسة نستطيع ان تستنتج ان غذاء النحل للنحل المسمى stingless bee التي تم الحصول عليها من مناطق مختلفة يحوي بكتريا حامض اللبنيك مع فعالية مضادة للميكروبات. مع خصائص بروبيوتك متنوعة و اقل تاثيرا لبعض المضادات الحيوية.

الكلمات المفتاحية للبحث: غذاء النحل، بكتريا حامض اللبنيك، خصائص البروبيوتك، الفعالية المضادة

للجراثيم

## ABSTRACT

Information relating to the types of lactic acid bacteria (LAB) present in stingless bee bread farm in Malaysia, their antimicrobial and probiotic properties is lacking. Bee bread is rich in nutrients, acidic in nature and may support growth of many microorganisms including LAB. The purpose of this study was to isolate lactic acid bacteria from bee bread of stingless-bee obtained from different stingless bee species and sources. The isolated LAB were evaluated for their antibacterial activity against three pathogenic bacteria (*Staphylococcus aureus* ATCC 25923, *Salmonella* Typhimurium ATCC 13311 and *Escherichia coli* ATCC 25922) and, the probiotic properties namely, tolerance to low pH, and survival in 0.1 to 0.5% bile-salt and antibiotic resistant. Pre-enrichment of bee bread in MRS broth facilitate isolation of fifteen LAB strains on MRS agar with 0.8% CaCO<sub>3</sub> compared to tomato juice agar with 0.8% CaCO<sub>3</sub>. The isolates were assured LAB by negative catalase test and Gram positive. LAB isolates showed significant ( $p < 0.05$ ) inhibitory activity against targeted bacteria. LAB isolated from *Thorasica* spp., *Itama* spp. and *Terminata* spp. isolated from Negeri Sembilan (NS) was more effect against *S. Typhimurium* and *E. coli* while LAB isolated from Sungai Merab (SM) showed the highest inhibitory activity against *S. aureus* as indicated by the diameter of inhibition zone: *S. aureus* (14.0-25.8 mm), *Salmonella typhimurium* (23.1 to 37.6 mm) and *Escherichia coli* (17 to 39.6 mm) after 24 h incubation at 37°C by dual agar overlay method. Supernatants of all LAB isolates showed an excellent antibacterial activity against all target bacteria at 50% of the cell free supernatant. Loss of antibacterial activity was observed after adjustment of supernatant to pH 5, 6, 7 and 8. All LAB isolates survived in 0.1 to 0.5 % (w/v) bile-salt and pH 2 to 4. The LAB isolated from bee bread SM were more resistant to high pH and bile-salt than LAB isolated from bee bread NS. All LAB isolates were resistant to vancomycin (5 µm) and streptomycin (10 µm); LAB from bee bread SM were resistant to gentamycin (10 µm) while LAB from bee bread NS were less resistant. All LAB isolates were sensitive to tetracycline (30 µm), penicillin G (10 µm) and chloramphenicol (30 µm). It can be concluded that bee bread of stingless bees obtained from different sources contains LAB with good antimicrobial activity against the target pathogens, but variable probiotic properties and less susceptibility to some antibiotics.

Keywords: *Bee bread, lactic acid bacteria, probiotic properties, antimicrobial activity, stingless bees*



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## ABBREVIATIONS

LAB	Lactic acid bacteria
SM	Sangai Merab
NS	Negeri Sembilan
K	Kedah
T	Terengganu
T - SM	<i>Thorasica</i> spp. - Sungai Merab
T - NS	<i>Thorasica</i> spp. – Negeri Sembilan
I - NS	<i>Itama</i> spp. - Negeri Sembilan
Te - NS	<i>Terminata</i> spp. - Negeri Sembilan
MRS	De Man Rogosa and Sharpe
TJA	Tomato juice agar
CaCO <sub>3</sub>	Calcium carbonate
NA	Nutrient agar
CFS	Cell free supernatant
Cfu/ml	Colony forming units per millilitre
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
μl	Microliter
mm	Millimetre
w/v	Weight per volume
%	Percentage
OD	Optical Density